5'-GTACTAGTAGTTCCTGCTATGT-3' (SEQ ID NO: 40) and plasmid pCMVΔR8.74 to amplify a 298 bp fragment containing nucleotides 1215 through 1513 of HIV-1 HXB2 (Genbank accession number K03455). Probe 2 was generated by PCR using primers 5'-CTGCTGACATCGAGCTTGCTACA-3' (SEQ ID NO: 41) and 5'-CTAGCTCCCTGCTTGCCCATACT-3' (SEQ ID NO: 42) and plasmid pHR2 as template to amplify a 577 bp fragment containing nucleotides 336 through 913 of HIV-1 HXB2 (Genbank accession number K03455). ^{32}P antisense riboprobe then was synthesized by T7 RNA polymerase in the presence of UTP (800Ci/ml, DuPont NEN™). Full length probes were gel purified and stored in 0.5 M ammonium acetate, 1 mM EDTA, and 0.2% SDS elution buffer at-20°C. RNA protection assay was performed using a HybSpeed™ kit (Ambion) according to manufacturer instructions. rnase A/T1 mix (0.5 U/20 U per reaction, Ambion) digestion protected probe fragments were separated on 4% polyacrylamide, TBE and 8 M urea gels. For fragment size determination, ^{32}P-labeled an RNA markers were synthesized on RNA Century template set and electrophoresed in parallel. For band detection and intensity quantification, dried gels were exposed either to photofilm or a phosphorimager plate (Molecular Dynamics).

A9 cont.

Example 10

Transfer Vector Constructs. pHR'CMV-LacZ and pHR'CMV-Luciferase have been described (Naldini et al., Science, supra). pHR2 is a lentiviral transfer vector in which the polylinker and downstream nef sequences up to the KpnI site of pHR' have been replaced with a ClaI/SpeI/SnaBI/SmaI/BamHI/SacII/EcoRI polylinker. pHR2 was generated by replacing the 3.7 kb ClaI-SacI fragment of pHR'CMVlacZ with a 607 bp ClaI-SacI fragment generated by PCR using pHR'CMVlacZ as the template with oligonucleotide primers 5'-CCATCGATGGACTAGTCCTACGTATCCCCGGGGACGGGATCCGCGGAATTCC GTTTAAGACCAATGAC-3' (SEQ ID NO: 43) and 5'-TTATAATGTCAAGGCCTCTC-3' (SEQ ID NO: 44), followed by digestion with ClaI and SacI.

Please replace the paragraphs at page 47, line 8, to page ~~49~~ 48, line 5, with the following: